



Chlorophyll

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Chlorophyll Sampling

- Sample Collection
- Sample Handling
- Filtration and Storage
- Extraction
- Analyses

Sample Collection

- Where to sample in the water column
 - Photic zone
 - Light penetration
 - Stratified waters
- How to sample
 - Grab samples
 - Discrete depth
 - Depth integrated
- In Situ sampling

Sample Handling

- Field: Whole water samples should be kept cold (4°C) and in the dark; filtration in the field is not necessary and is not recommended.
- Laboratory: Prior to filtering and extraction, samples may be kept cold (4°C) and dark for up to two days, although more immediate filtration is recommended to prevent bottle effects.
- Records of field and laboratory handling (that all important metadata) are imperative.

Filtration and Storage

- Whole water samples should be filtered or centrifuged to concentrate. Filtration on a 45 μ pore size membrane filter or small pore size (GF/F) glass fiber filter is preferred to centrifugation.
- Filtration should be conducted with low pressure to prevent cell breakage during filtering.
- Filters may be stored frozen in the dark for up to three weeks. (Acidic water samples should be analyzed immediately.)

Extraction

- Extraction of chlorophyll is accomplished using 90% aqueous alkaline acetone.
- Frozen filters are ground in a tissue grinder with acetone solution for extraction, this slurry solution is then centrifuged or filtered to clarify the extractant.
- Filtration (previous slide) and extraction should be conducted in low light to prevent excitation and subsequent degradation of the chlorophyll before analysis.

Analyses

- Chlorophyll is measured by determining the intensity of excitation at a specific wavelength.
- Wavelengths used vary based on the analytical method (trichromatic, chlorophyll a, acid corrected chlorophyll a)
- Pheophytin a, a chlorophyll degradation product, mimics chlorophyll a and can cause an overestimation of chlorophyll. The amount of pheophytin a present can be estimated by acidifying the chlorophyll sample and converting all chlorophyll to pheophytin.
- Fluorometric analyses is preferred to spectrophotometric analysis in marine/estuarine waters.

Analytical Choices

- Trichromatic method: is useful when identifying dominance of different algal groups.
- Acid-corrected method: is most commonly used.
- Fluorometric versus Spectrophotometric:
 - fluorometric is more sensitive;
 - spectrophotometric is more commonly used, especially historically;
 - fluorometric is not recommended in freshwaters high in organic acids (blackwaters) due to problems with interference; fluorometric is more sensitive.

So What?

- Consistency is key
- Whatever you do, do it consistently
- Analytical choices are less important than replicable sample collection, storage, and processing.